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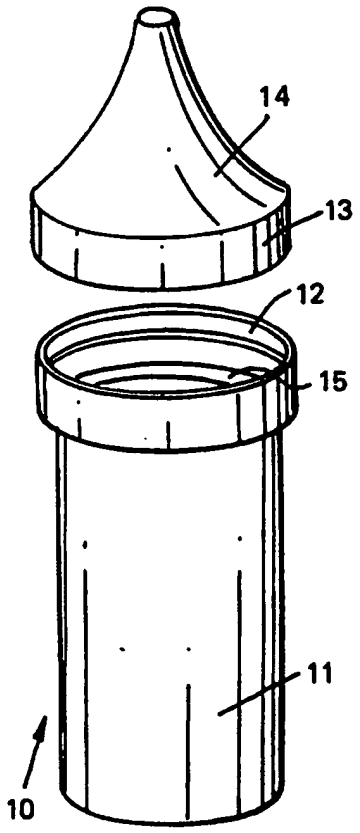
(54) Title: DEVICE FOR THE PROCESSING OF SALIVA FOR USE IN AN IMMUNOASSAY

(57) Abstract

A device (10) is provided for the processing of collected saliva for direct use in an immunoassay. The device (10) suitably comprises a receptacle (11) with flexible walls for receiving an amount of saliva. The receptacle (11) is inter-engageable with a filtration device (13) which has an integral dropper mechanism (14). The filtration device (13) has a number of layers of a filter material having a gradation of pore sizes which permit passage of immunoglobulins while retaining mucous, tissue and food debris contained in saliva. An absorbent pad (17) mounted on a handle (18) can be used to collect saliva and transfer it to the receptacle (11), whereupon handle (18) is snapped off at a weakened line (19). Squeezing of the receptacle (11) facilitates release of immunoglobulins into a buffer whereupon the device (10) is inverted so as to allow filtration of the saliva to take place.



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Description

Device for the processing of saliva for use in an immunoassay

Technical Field

This invention relates to the collection and processing of saliva for use in an immunoassay. More particularly, the invention relates to a device for the processing of collected saliva for direct use in an immunoassay and additionally to means for collecting the saliva to be processed.

Background Art

The oral immune system interacts actively with the immune system of the rest of the body. Within the oral cavity there is found a specific site of antigen-antibody response which involves extraoral lymph nodes and intraoral lymphoid aggregations.

By saliva herein is included gingival liquid, hereinafter referred to collectively as saliva.

The intraoral lymphoid tissue comprises essentially four distinct tissue aggregations: the gingival lymphoid tissue; salivary gland lymphoid tissue; scattered sub-mucosal lymphoid aggregations; and the tonsils.

The salivary glands produce and secrete through their plasma cells predominantly IgA. The IgA antibodies produced by the salivary glands are referred to as the secretory IgA and they represent the major immunoglobulin fraction contained in saliva.

The presence of IgG and secretory IgA in the saliva prompted the use of saliva as a basis for immunological assays. The suitability of saliva as a diagnostic body fluid has not been demonstrated for all diseases or even a significant proportion thereof. Recent studies have

demonstrated that saliva can be successfully used in testing for various conditions and diseases, including HIV antibody detection, rubella and hepatitis.

If one is to successfully use saliva as a diagnostic body fluid, several key factors must be taken into consideration: a) the type of antibody to be detected or determined; b) assay sensitivity; and c) the method of saliva collection.

As indicated above, IgA antibodies are the major immunological fraction contained in saliva. In many instances, there are not enough specific IgA antibodies in saliva to perform a specific diagnostic test. Furthermore, the specific IgA may not be present in a sufficient amount to perform a test that compares favourably with a corresponding blood test. An example of the latter is the case of HIV1 antibodies.

It is known that saliva contains about 0.01-0.1% of the immunoglobulins contained in the blood. Because of the reduced immunoglobulin content of saliva relative to blood, it is necessary to use more sensitive antigen-antibody assay methods relative to corresponding assays performed on the blood or blood fractions.

The collection of saliva is a relatively complicated procedure and can lead to misleading results when the saliva is collected from the salivary glands because of the relatively small volumes that can be collected and because of the viscosity and high mucous content of saliva. Most of the methods currently employed involve collecting saliva through capillary tubes or suctioning saliva into micro-pipettes or syringes. The use of saliva collected by these methods has inherent problems because of the high content of mucous and the limitations presented by the predominance of IgA, which is known not to be present in a sufficient amount in the case of certain specific antibodies.

In recent years much attention has been directed to the use of saliva for diagnostic purposes and new methods of saliva collection

have been developed to collect IgG antibodies. Many of the new methods involve the absorption of saliva in the mouth by an absorbent material from which the collected saliva is extracted and processed. With such methods a number of potential problem areas arise: i) the volume of sample collected; ii) the manipulation of the collected sample; iii) the mucous and tissue content of the collected sample; iv) the separation of the immunoglobulins from the mucous, tissue and saliva; v) the preservation or non-preservation of the immunoglobulins; vi) patient comfort during collection; and vii) the type of immunoglobulins collected.

One major advantage of the use of saliva as a body fluid for diagnostic assays is that the taking of saliva samples is non-invasive as compared with the taking of blood samples. Thus, the collection of saliva for such assays can be carried out in situations where the availability of syringes might be limited or where strict hygiene practices might not be adhered to.

Thus, the use of saliva as a basis for diagnostic assays is desirable. However, the processing of saliva once collected currently involves the use of laboratory equipment and instrumentation, such that testing 'on the spot' cannot normally be carried out. Therefore, there is a need for a method of collecting saliva and processing the saliva once collected that can be performed at the site of collection, for direct use in a diagnostic assay.

Disclosure of Invention

The invention provides a device for the processing of collected saliva for direct use in an immunoassay, comprising a receptacle for receiving an amount of saliva, said receptacle being inter-engageable with a filtration device at the free end thereof, such that inversion of the receptacle with the filtration device in position enables collected saliva to be filtered and the filtrate obtained to be directly used in an immunoassay for an immunoglobulin of interest.

The device according to the invention allows one to collect saliva from the oral cavity in sufficient amounts and in a form for direct use in an immunoassay, without a requirement for the usual laboratory instrumentation for the processing of the saliva once collected, as hereinafter described.

The invention also enables one to obtain a saliva sample suitable for use in an on the spot, rapid immunoassay with a minimum of sample manipulation following collection thereof.

Preferably, the receptacle has a capacity of less than 10 ml, more especially 2-3 ml.

Preferably, the filtration device comprises one or more layers of a filter material having a gradation of pore sizes in the range 0.5-5 μm.

Further, preferably, the final layer through which the saliva percolates should have a pore size no greater than 1.0 μm.

Suitable materials for use in the filtration device include layers of glass, ceramic, wood, paper, etc., more especially glass.

The filtration device preferably has an integral dropper mechanism, so that the filtrate can be transferred in droplets of a predetermined size, when desired.

In one embodiment the saliva is collected by means of an absorbent pad wiped over a surface in the oral cavity, said absorbent pad being formed of a material which has a low binding capacity for immunoglobulins, and said absorbent pad being receivable in the receptacle of the device.

Where the saliva is collected by means of an absorbent pad, the walls of the receptacle are preferably flexible, whereby the application of pressure to said walls facilitates release of immunoglobulins from

the absorbent pad by causing agitation of a liquid medium contained in the device.

The flexible receptacle is preferably formed of a flexible plastics material. Suitably the receptacle has a tubular shape approximately 30 mm in length and with a diameter of approximately 15 mm.

The receptacle is also suitably provided with a cap or other closing means.

The absorbent pad when such is used to collect saliva is preferably mounted at the end of a handle to facilitate the collection of saliva and the transfer thereof, once collected, to the receptacle. Indeed, the absorbent pad and associated handle can resemble a device commonly used in the home and referred to as a cotton bud or tip or as sold under the Trade Mark Q-TIP.

The absorbent pad can be of any suitable shape which permits the collection of saliva, when the pad is wiped over a surface in the oral cavity such as the gums or under the tongue for approximately 2 minutes. At the end of the collection period, the saliva impregnated pad is removed from the mouth and transferred to the receptacle for processing of the saliva.

The absorbent pad is suitably made of cotton, paper, rayon, or sponge or other material which has a low binding capacity for immunoglobulins. An especially suitable material is rayon. The use of rayon material for the absorbent pad eliminates a common problem observed with immunoglobulins which is non-specific binding.

Thus, using a rayon absorbent pad or other material with a very low binding capacity for immunoglobulins obviates the need for the addition of agents to the absorbent material to prevent non-specific binding, which agents can cause problems for the patient when the saliva collection pad is inserted in the oral cavity. Furthermore, the

use of such agents can lead to problems as regards obtaining regulatory approval for a saliva collection device.

It has been found that rayon material has a high absorption capacity, allowing the collection of 0.1-1.1 ml of saliva, when used to collect saliva as hereinbefore described. The absorbent pad is suitably 10-30 mm in length and 5-15 mm in diameter.

The handle suitably has a length of 60-100 mm and a diameter of 1-3 mm.

The handle is preferably provided with a weakened line adjacent the absorbent pad which facilitates detachment of the handle once the absorbent pad has been inserted in the receptacle for processing of the collected saliva. Once the pad has been inserted in the receptacle, the handle is broken at the weakened line through a snapping action. More specifically, the absorbent pad is inserted into the receptacle until the weakened line of the handle lines up with the top edge of the receptacle, the top edge providing resistance for said handle. Once the handle is detached, leaving the pad inside the receptacle, the handle is discarded.

When an absorbent pad is used to collect saliva, an amount of a suitable buffer, such as phosphate buffered saline (PBS) at pH 7.2, is inserted in the receptacle, such that once the absorbent pad is inserted in the receptacle, the buffer will saturate said pad, releasing antibodies therefrom. By squeezing the receptacle several times, the antibodies will equilibrate between the respective phases. Suitably, the receptacle contains approximately 0.75 ml of PBS.

Following equilibration, the receptacle is inverted with the filtration device mounted at the free end and immunoglobulin material, free of mucous, tissue and food debris resulting from the filtration process is obtained in a form ready to use in an immunoassay.

It has been found that the use of the aforementioned absorbent materials with low binding capacities enables one to collect and process

saliva so that one obtains amounts of antibodies in a sufficient concentration to perform an immunoassay, as hereinafter described.

The dropper mechanism when used in association with the filtration device suitably provides drops of processed liquid of approximately 30-50 µl.

As an alternative to the use of an absorbent pad for the collection of saliva, a rinse solution may be used.

A suitable rinse solution is PBS at a pH of 7.2.

Thus according to an alternative embodiment of the invention approximately 5 ml of sterile PBS is used to rinse the mouth for approximately 1-2 minutes. After the rinsing operation, the solution is expelled by the patient into a container from which the PBS was administered. Approximately 2 ml of the collected solution is transferred into the receptacle in accordance with the invention. In this embodiment also, the walls of the receptacle are preferably flexible.

The collected salivary material is filtered through the filtration device in the same manner as material released from the absorbent pad. Thus, the receptacle is inverted to allow the collected material to percolate through the filtration device for the removal of mucous, tissue and food debris, to provide a sample or specimen which can be immediately used in an immunoassay.

Thus the filtration of collected saliva in accordance with the invention provides a sample which is free of entrained mucous, tissue and food debris, which can be used without any further manipulation.

Brief Description of Drawings

The invention will be further illustrated by the following description of an embodiment thereof given by way of example only with reference to the accompanying drawings in which: -

Fig. 1 is an exploded schematic representation of a device according to the invention for the processing of collected saliva; and

Fig. 2 is a schematic representation of a saliva collection device for use with the device of Fig. 1.

Best Mode for carrying out the invention

Referring to Fig. 1, there is illustrated a device indicated generally at 10, for the processing of collected saliva. The device 10 comprises a tubular receptacle 11, 30 mm long and 15 mm in diameter and made of a flexible plastics material. The receptacle 11 receives at its open end 12, a filtration device 13 with an integral dropper mechanism 14. The filtration device 13 can be inter-engageable with the receptacle 11 in known manner and in such a way that the device is sealed against any egress of the sample when the device 10 is in an inverted mode, other than through the filtration device 13 and the associated dropper mechanism 14. The filtration device 13 in the engaged position abuts an internal rib 15 provided on the receptacle 11.

Referring to Fig. 2, there is indicated generally at 16, a saliva collection device having an absorbent pad 17 of rayon material mounted on a plastics handle 18. The absorbent pad 17 is 15 mm long and 10 mm in diameter. The handle 18 is 60 mm long and is provided with a weakened line 19 adjacent the absorbent pad 17.

In use, the device 16 is used to collect a sample of saliva on the absorbent pad 17 from the oral cavity of a patient as hereinabove described.

The device 16 is used to transfer a collected sample to the device 10 which contains an amount of a buffer such as PBS, suitably 0.75 ml, at pH 7.2. The device 16 is inserted into the receptacle 11 to an extent such that the weakened line 19 is adjacent the upper edge thereof, whereupon the handle 18 is broken off by a snapping action and

discarded. The filtration device 13 and integral dropper mechanism 14 is attached to the device 10 and the walls of the device 10 are squeezed so as to facilitate release of bound antibody into the PBS buffer. The device 10 is then inverted, whereupon the contents of the device percolate through filtration device 13, assisted by further squeezing of the tubular body 11. The filtration device 13 has a number of layers of a filter material (not shown) as hereinabove described with graded pore sizes which retain mucous, tissue and food debris contained in saliva while permitting immunoglobulins to pass therethrough into the dropper mechanism 14 for collection. Droplets of filtrate are collected from the dropper mechanism 14 for use in an immunoassay.

Example

Ten patients known to be positive for HIV antibodies in their blood were tested. Saliva was collected using a device 16 of the type illustrated in Fig. 2 and the saliva processed using a device 10 of the type depicted in Fig. 1.

Two tests specific for HIV antibodies were run in parallel as follows: a) Western blot; and b) SalivaCard - rapid assay.

The SalivaCard (Trade Mark) assay is a modified version of the SeroCard (Trade Mark) assay - marketed in Canada and the U.S.A. by Disease Detection International, Inc., Irvine, California, U.S.A. and manufactured and marketed in countries other than Canada and the U.S.A. by Trinity Biotech plc - modified to run on saliva instead of serum.

All ten patients were found to be positive for HIV antibodies by both methods. One patient had only a 160 band response by Western blot, but was found to be positive by the SalivaCard assay.

Ten known HIV negative patients were also tested in the same

manner as for the HIV positive patients and again all ten results were found to be negative by both methods.

* * * *

Thus, it will be appreciated that the collection and processing of saliva in accordance with the invention is an effective method for providing samples by a non-invasive method for use in immunoassay.

It will be appreciated that the device according to the invention has particular application in poorer areas of the world, such as Third World countries, where access to laboratory instrumentation may not be a reality. The device according to the invention has particular application for the testing of patients for AIDS and thus its advantages are clear as regards Third World countries such as countries in the African Continent where AIDS is pandemic, but also for on the spot checking at frontiers.

Claims

1. A device for the processing of collected saliva for direct use in an immunoassay, comprising a receptacle for receiving an amount of saliva, said receptacle being inter-engageable with a filtration device at the free end thereof, such that inversion of the receptacle with the filtration device in position enables collected saliva to be filtered and the filtrate obtained to be directly used in an immunoassay for an immunoglobulin of interest.

2. A device according to Claim 1, wherein the receptacle has a capacity of less than 10 ml.

3. A device according to Claim 1 or 2, wherein the filtration device comprises one or more layers of a filter material having a gradation of pore sizes in the range 0.5-5 μm.

4. A device according to Claim 3, wherein the final layer through which the saliva percolates has a pore size not greater than 1 μm.

5. A device according to any one of Claims 1-4, wherein the filtration device has an integral dropper mechanism, so that the filtrate can be transferred in droplets of a predetermined size, when desired.

6. A device according to any one of Claims 1-5, wherein the saliva is collected by means of an absorbent pad wiped over a surface in the oral cavity, said absorbent pad being formed of a material which has a low binding capacity for immunoglobulin, and said absorbent pad being receivable in the receptacle of the device.

7. A device according to Claim 6, wherein the walls of the receptacle are flexible, whereby the application of pressure to said walls facilitates release of immunoglobulins from the absorbent pad by causing agitation of a liquid medium contained in the device.

8. A device according to any one of Claims 1-5, wherein the saliva is collected by means of a rinse solution which is used to rinse the oral cavity and which is receivable in the receptacle of the device after rinsing.

9. A device according to any preceding claim, wherein the walls are formed of a flexible plastics material.

10. A device according to any preceding claim, wherein the receptacle is provided with closing means.

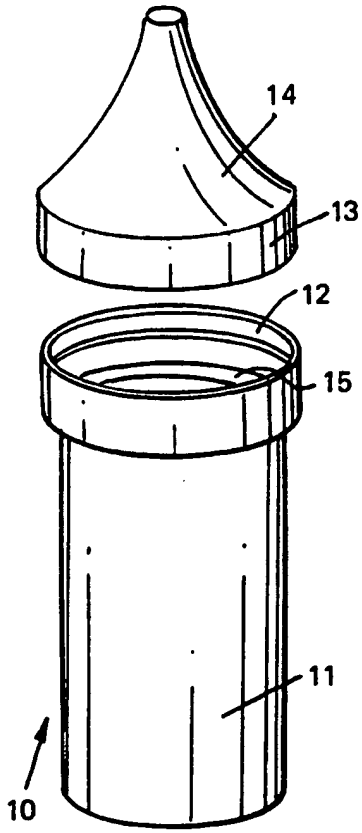

FIG. 1

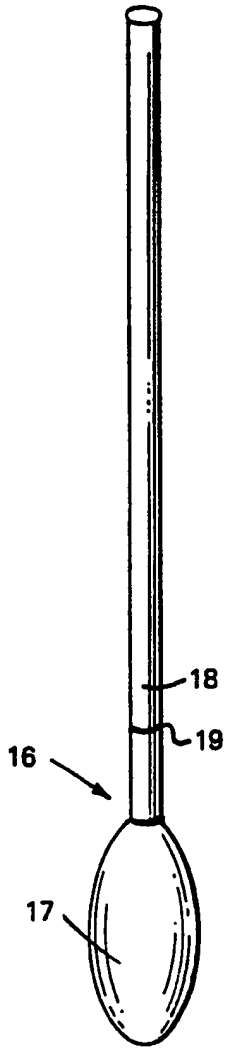

FIG. 2

INTERNATIONAL SEARCH REPORT

Int ...nal Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61B10/00 B01L3/02 B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61B B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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